Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Light-nutrition Coupling Effect of Degradable Fluorescent Carbon Dots on Lettuce

*Xiaoqin Pan,[a,b] Fangmei Fu,[a] Zhenxi Xie,[a] Wei Li,[a,c] Xian Yang, [b] Yunyan Kang, [b,c] Songnan Qu,[d] Yinjian Zheng,[e] Qingming Li,[e] Haoran Zhang,[a,c] Shiwei Song,[*b] and Bingfu Lei,[*a,b,c]*

[a] Guangdong Provincial Engineering Technology Research Center for Optical Agriculture, College of Materials and Energy, South China Agricultural University, Guangzhou 510642, P. R. China

[b] College of Horticulture, South China Agricultural University, Guangzhou 510642, P. R. China

[c] Maoming Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Guangdong Maoming 525100, P. R. China

[d] Institute of Applied Physics and Materials Engineering, University of Macau, Taipa, Macau, P. R. China

[e] Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu 610213, P. R. China

[*]Corresponding authors: E-mail addresses: swsong@scau.edu.cn (S. Song), tleibf@scau.edu.cn (B. Lei, **Lead contact**).

EXPERIMENTAL DETAILS

Materials

All the reagents in this study (except HCl) were in analytical reagent grade that purchased from Macklin company.

CDs preparation

Table S1 reagent ratio for preparing different CDs

Sample	CA(g)	EDA (g)	HCl (mL)	Water (mL)
CA−CDs	3	0	0	50
N1−CDs	3	1	0.1	48.9
N3−CDs	3	3	0.3	46.7

Liquid Chromatography-Mass Spectrometry

LC-MS was conduct on Thermo Scientific Q Exactive Ultimate 3000 UPLC instrument. MS: Spray Voltage at 3200 v; Capillary Temperature at 300.00℃; Sheath Gas with 40.00 Arb; Aux Gas with 8.00 Arb; Max Spray Current at 100.00 μA; Probe Heater Temp. at 300.00 ℃; Ion Source is ESI+/-MS. LC: Column is Hypersil GOLD 100*2.1mm and 3um Thermo Scientific; Temperature at 25℃; Wavelength from190 to 400 nm. U=0.25ml/min; Mobile phase including (A) methanol and (B) aqueous solution of 0.1% formic acid; Gradient elution: t=0-4-12-12.1-20 A%=5-5-70-70-5-5.

·OH elimination and production.

Salicylic acid was used as chromogenic agent for detecting ·OH by recording the absorption at OD_{510}[1]. For ·OH elimination, 1 mM H_2O_2 was mixed with 0.9 mM $FeSO_4$ to generate ·OH. Subsequently, different CDs with concentration of 100 mg/L were added and 0.9 mM salicylic acid was added as chromogenic agent. After 30 min, OD_{510} was recorded. The elimination rate was calculated by the followed formula.

$$Eliminated\ rate\ (\%) = \frac{A_c - (A_j - A_i)}{A_C} \times 100\%$$

A_c: H_2O_2 + $FeSO_4$ + salicylic acid at OD_{510}

A_j: H_2O_2 + $FeSO_4$+ CDs + salicylic acid at OD_{510}

A_i: $FeSO_4$+ CDs + salicylic acid in dark at OD_{510}. (A_i in different light environment also recorded as evaluation of ·OH production)

CO_2 gas chromatograph

1 mL medical needle was used to extract 1mL gas sample from CDs for CO_2 content determination, with three repetitions. The instrument is GC-7900 chromatograph (Shanghai, Tianmei). The working conditions: nitrogen as carries gas, pressure at 0.5 Mpa; hydrogen as gas, pressure at 0.2-0.25 Mpa; air as auxiliary gas, pressure at 0.4 Mpa; column temperature at 80℃, injection pot temperature at 100℃, thermal conductivity temperature at 150℃,current at 300 mA. D-7900P Chromatograph workstation software was used to analyze the CO_2 content.

Isolation of the chloroplasts

The lettuce leaves were homogenized in a sucrose phosphate buffer that contained 0.40 M of

sucrose, 10 mM of KCl, 30 mM of Na_2HPO_4, and 20 mM of KH_2PO_4[2, 3]. Filtered through four layers of cotton gauze, the filtrates were subsequently centrifuged at 1000 rpm for 3 min. The supernatant liquid in the tube was centrifuged at 3000 rpm for another 3 min. The chloroplasts were obtained from the residue at the bottom of the tube and spread in a buffer solution into a final concentration of 400 $mg \cdot L^{-1}$

DCPIP reduction, ferricyanide reduction, and ATP formation

The chloroplast suspensions (200 $mg \cdot L^{-1}$) were respectively mixed with 0, 10, 50, and 100 $mg \cdot L^{-1}$ of an N−CDs buffer dispersion for 2 h.

For the DCPIP reduction experiment, prior to the illustration, 2.0 mL of a 120 μM DCPIP solution was added. Illuminated under a xenon lamp with an intensity of 7.0 $mW \cdot cm^{-2}$ for 5 min, the absorbance at 600 nm was recorded every 1 min.

For the ferricyanide reduction experiment, the N−CDs concentrations of 0 and 50 $mg \cdot L^{-1}$ were selected as the experimental groups. Before illustration, 100 μL of a 10 mM ferricyanide solution was added. They were then illuminated under a xenon lamp with an intensity of 7.0 $mW \cdot cm^{-2}$ for 2 min. Subsequently, the equivalent volume of 2% trichloroacetic acid was added[2]. The absorbance of the supernatant at 420 nm was recorded after 10 min of being centrifuged at 8000 g. The treatment group without irradiation was used as the dark tube to calculate the reduced rate.

For the ATP formation experiment, the groups with 0 and 50 $mg \cdot L^{-1}$ of N−CDs were used. Before illustration, 2.0 mM of the ADP solution was added[2]. After illuminating under a xenon lamp with an intensity of 7.0 $mW \cdot cm^{-2}$ for 2 min, an ATP Content Assay Kit was used to measure the ATP synthesis contents.

N−CDs treatments of lettuce.

When the lettuce grows to three leaves, the uniform lettuce is transferred to the plant culture shelf and cultured normally with Hogland nutrient solution. 10 days after, spraying water and 50 $mg \cdot L^{-1}$ N−CDs solution on different plants of lettuce leave as followed. Totally, 24 plant of lettuce required 15 mL of CDs solution every time.

Table S2 Treatment of the different groups

Groups	LED light (h)	UV-A light (h)	N3−CDs (50 $mg \cdot L^{-1}$)	Water
−CK	18	0	×	√
−50	18	0	√	×
+CK	18	4	×	√
+50	18	4	√	×

Laser confocal microscope

For the laser confocal microscope collection, thin slices of lettuce leave were observed using a laser confocal microscope (Zeiss LSM 710) at a 405 nm excitation. Lettuce with water treatment was control group, and the image of a 50 $mg \cdot L^{-1}$ N3−CDs solution treated lettuce leaf was collected.

Physiological indexes of lettuce

After 7 days of treatment, the Pn, E, Ci, and Gs were recorded using CIRAS-3 with a light intensity of 1000 $mol/(m^2 \cdot s)$.

Lettuce genes assay

The lettuce leaves were collected and sent to the BioMarker company for transcriptome sequencing and fluorescence qPCR after being treated for seven days. The differences in the RNA-seq data between the groups was compared using edgeR, a software package of the R language. The false discovery rate (FDR) and fold change (FC) were used as key indicators to screen differentially expressed genes. The screening conditions were FDR < 0.05 and | log2FC | > 1.2.

Details for qPCR

The reverse transcription was performed with the aid lab reverse transcription Kit (TUREscript 1st Stand cDNA SYNTHESIS Kit) and 18S was used as internal reference gene.

Table S3. **Primers for Real-time quantitative PCR**

Gene	Primer	Sequence (5'-3')
Reference gene	18S-F	CAACCATAAACGATGCCGA
	18S-R	AGCCTTGCGACCATACTCC
PEPC	100240-F	GAGCGGCGGAATAGAATC
	100240-R	AGACTGGGAGATGGAACC
MDH2	85260-1-F	TGGAGCACCTGGATTCAA
	85260-1-R	ACAAGCGGATTCATCTTCATT
GAPDH	11900-F	GCCTTATGACCACTGTTCACTCCA
	11900-R	GCTCTTCCACCTCTCCAATCCTT
ALDO	15680-F	GCGGTATCCTTGCCATTGAC
	15680-R	GCTTCGGTGTTGTCCAATCC
psbW	9761-F	TGTGTCATCATTGGTTGCTA
	9761-R	CCTTCCGTGCTCATTCTC

Chlorophyll content measurement

Soak the lettuce leaves in ethanol acetone mixture (1:1) for more than 48 hours, and then measure the absorbance values of extract at OD_{645}, OD_{663} and OD_{440}. Calculate the content according to the following formula,

$$C_{total}(mg/L) = 8.02 \times OD_{663} + 20.20 \times OD_{645}$$

$$Total\ chlorophyll\ content\ (mg/g) = (C_{total} \times V)/W$$

where C_{total} represent the total chlorophyll concentration, V represents the extract volume and W is the leave weight of lettuce.

SUPPLEMENTAL FIG.S

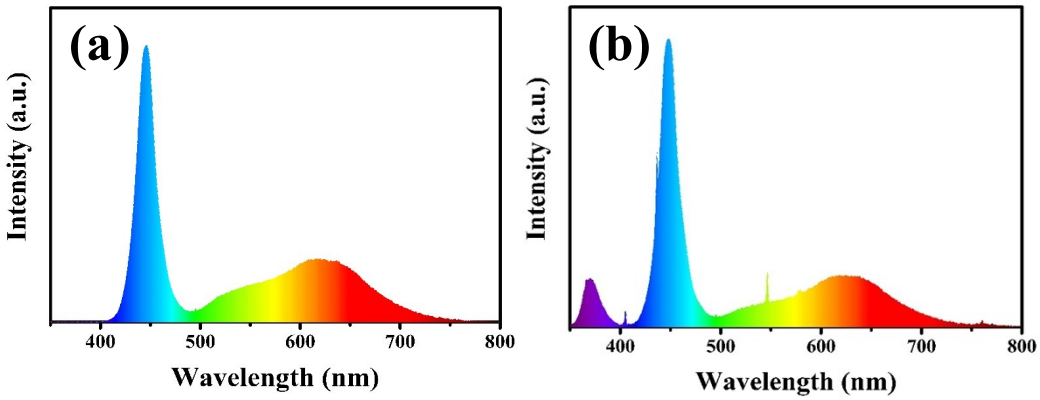

Fig. S1 Luminescence spectrum of lettuce cultivation conditions. (a) LED light; (b) LED plus with UV-A light.

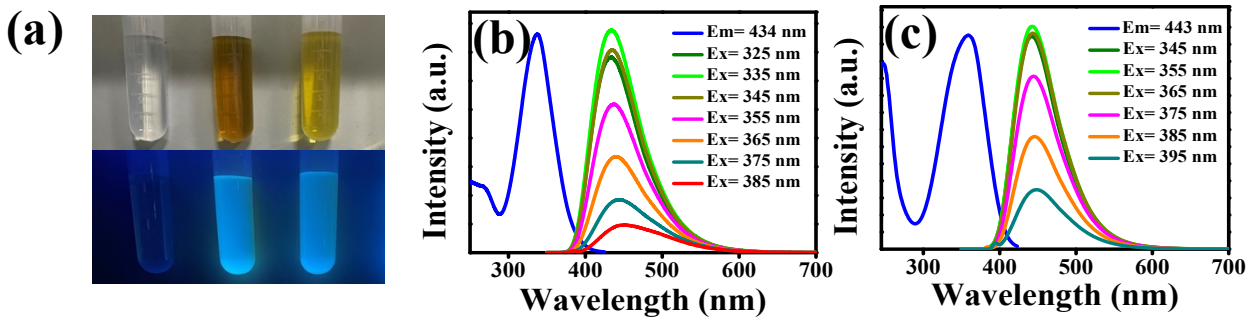

Fig. S2 (a) digital picture of three CDs under environmental light and 365 nm light (from left to right are CA-CDs, N1-CDs and N3-CDs). (b) PL spectra of N1-CDs. (c) PL spectra of N3-CDs.

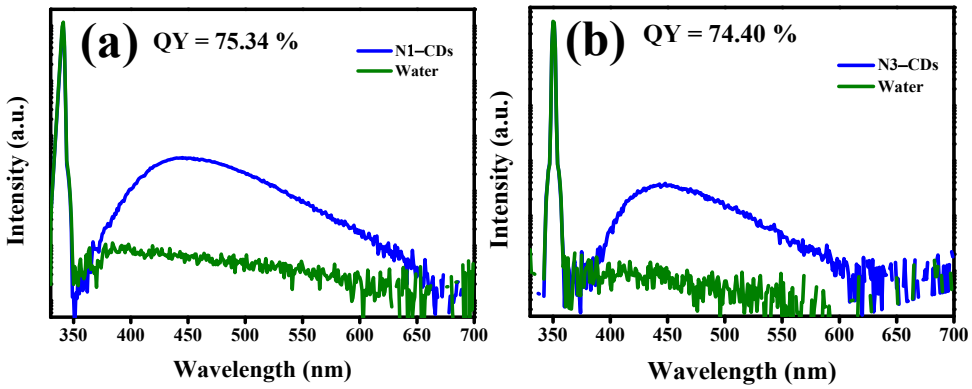

Fig. S3 Quantum yield spectra of N1−CDs (a) and N3−CDs (b) at 345, 350 nm excitation, respectively.

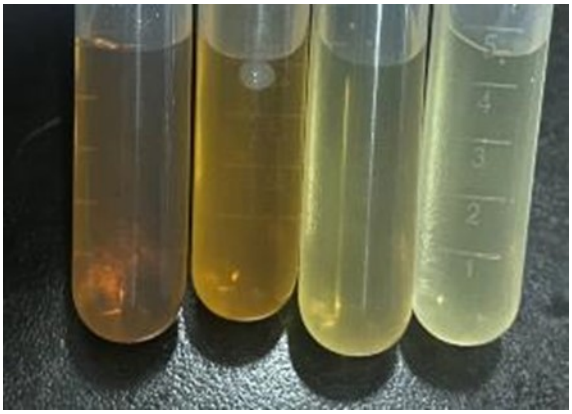

Fig. S4 Digital pictures of 1 mg/mL N-doping CDs (from left to right are N1-CDs in dark, N1-CDs after 7 days of LED+UV light, N3-CDs in dark, N3-CDs after 7 days of LED+UV light).

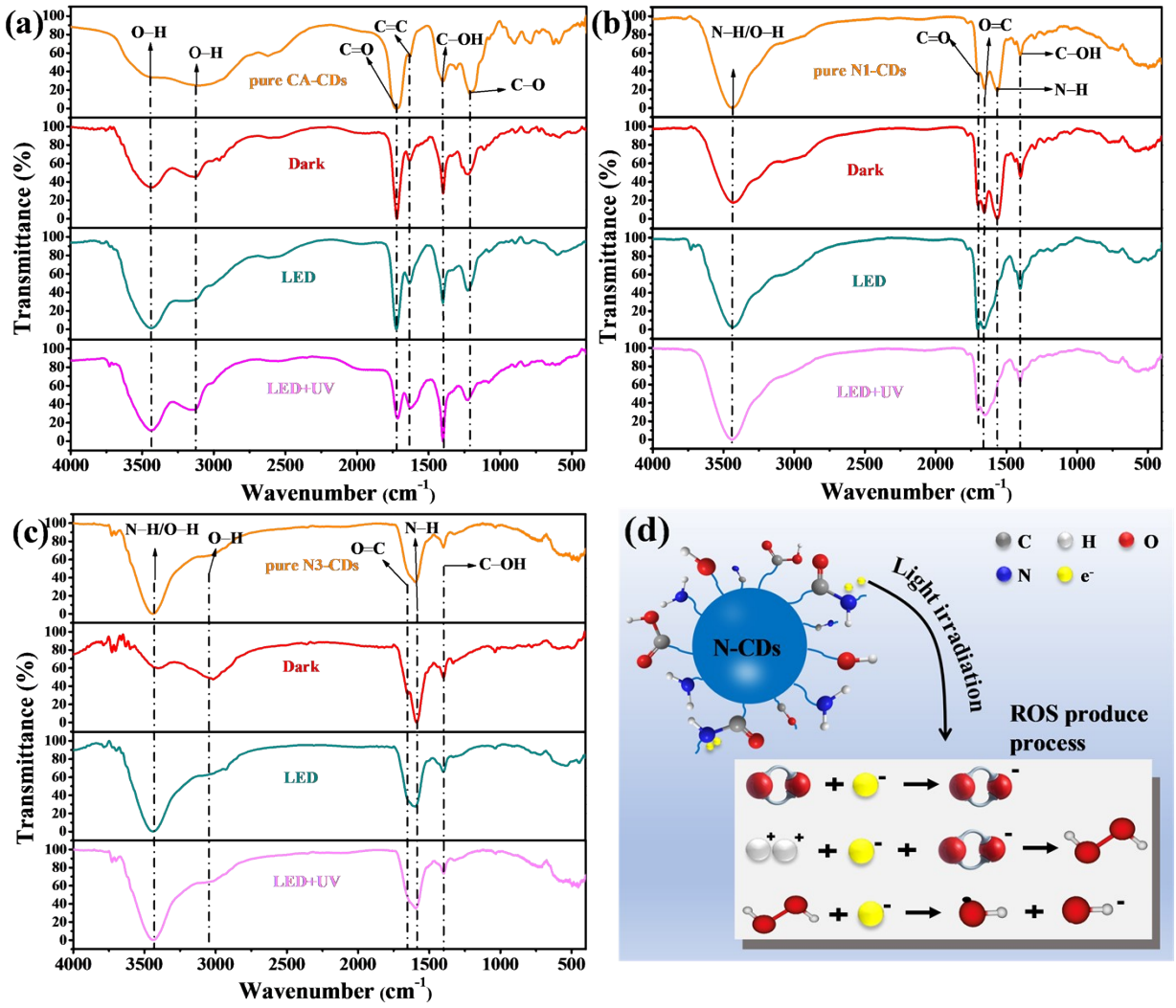

Fig. S5 FTIR spectra of CA−CDs (a), N1−CDs (b), N3−CDs (c), and ROS producing process diagram of N doping CDs (d).

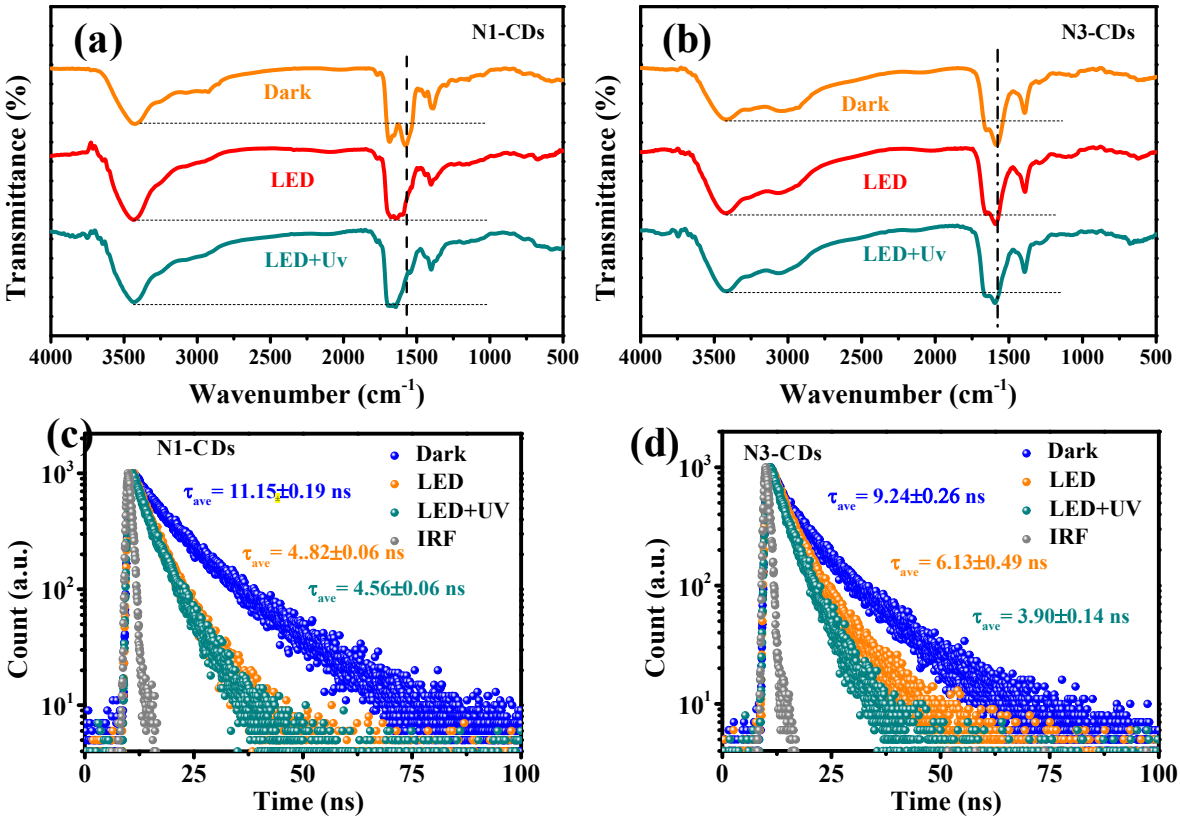

Fig. S6 FTIR spectra and fluorescence lifetime decay curves monitored at 454 nm and 434 nm emission of CDs after light in pure water after 7 days.

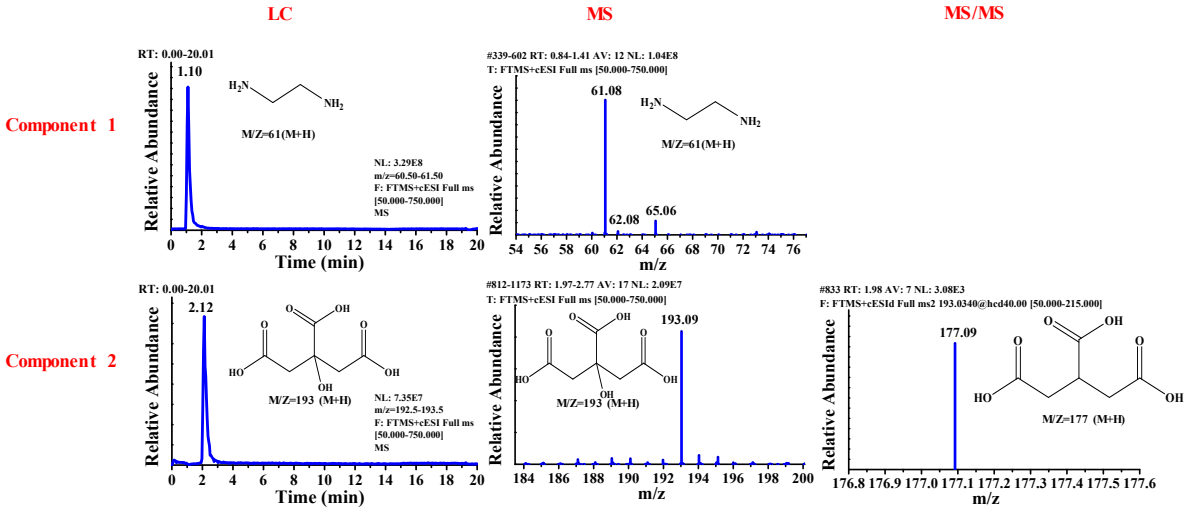

Fig. S7 LC-MS results of N3-CDs precursor mixture.

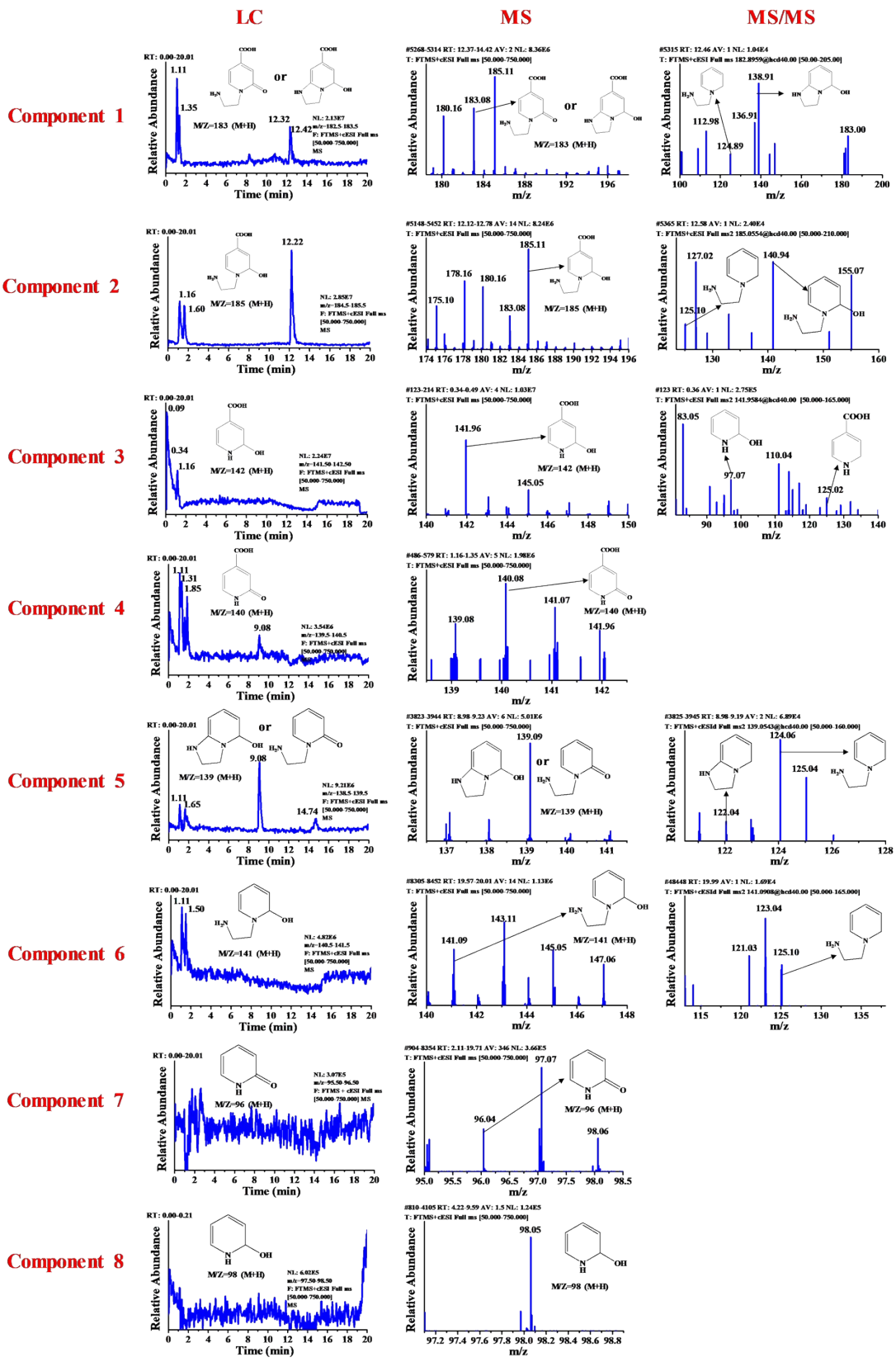

Fig. S8 LC-MS results of N3-CDs after degradation by H_2O_2 under 10 days of LED+UV light.

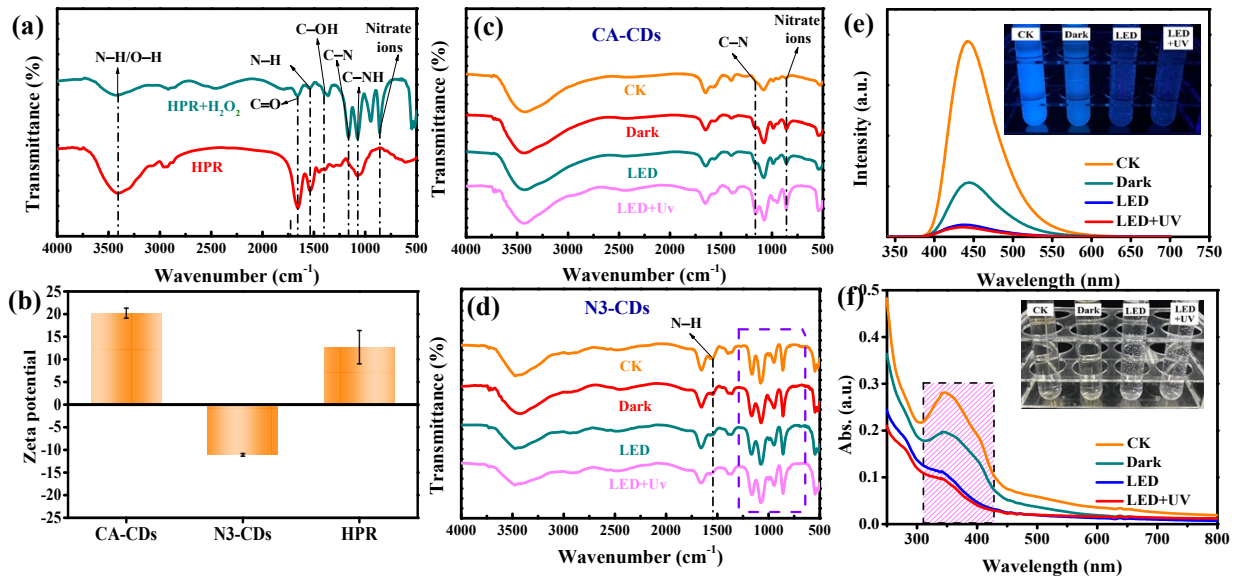

Fig. S9 (a)FTIR spectra of HPR, HPR incubated with H_2O_2 for 7 days; (b) Zeta potential of CA−CDs, N3−CDs and HPR; (c, d) FTIR spectra of CA−CDs and N3−CDs after 7 days incubation with HPR and H_2O_2 solution; (e, f) PL spectra and UV-vis absorption spectra of N3−CDs after 7 days incubation with HPR and H_2O_2 solution.

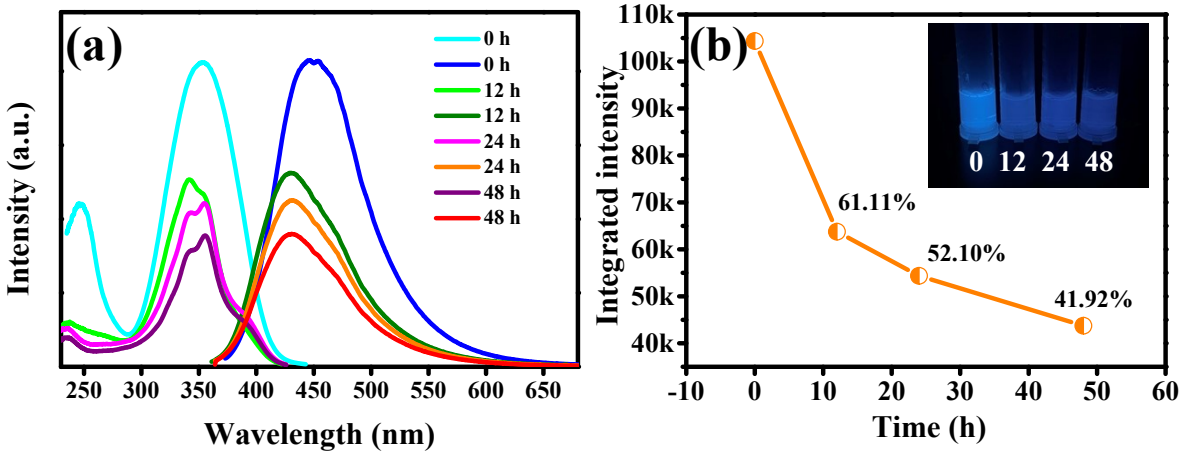

Fig. S10 PL spectra of N3−CDs during 2 days LED+UV illumination (a) and its integrated intensity evolution (b), inset: digital picture under 365 nm irradiation.

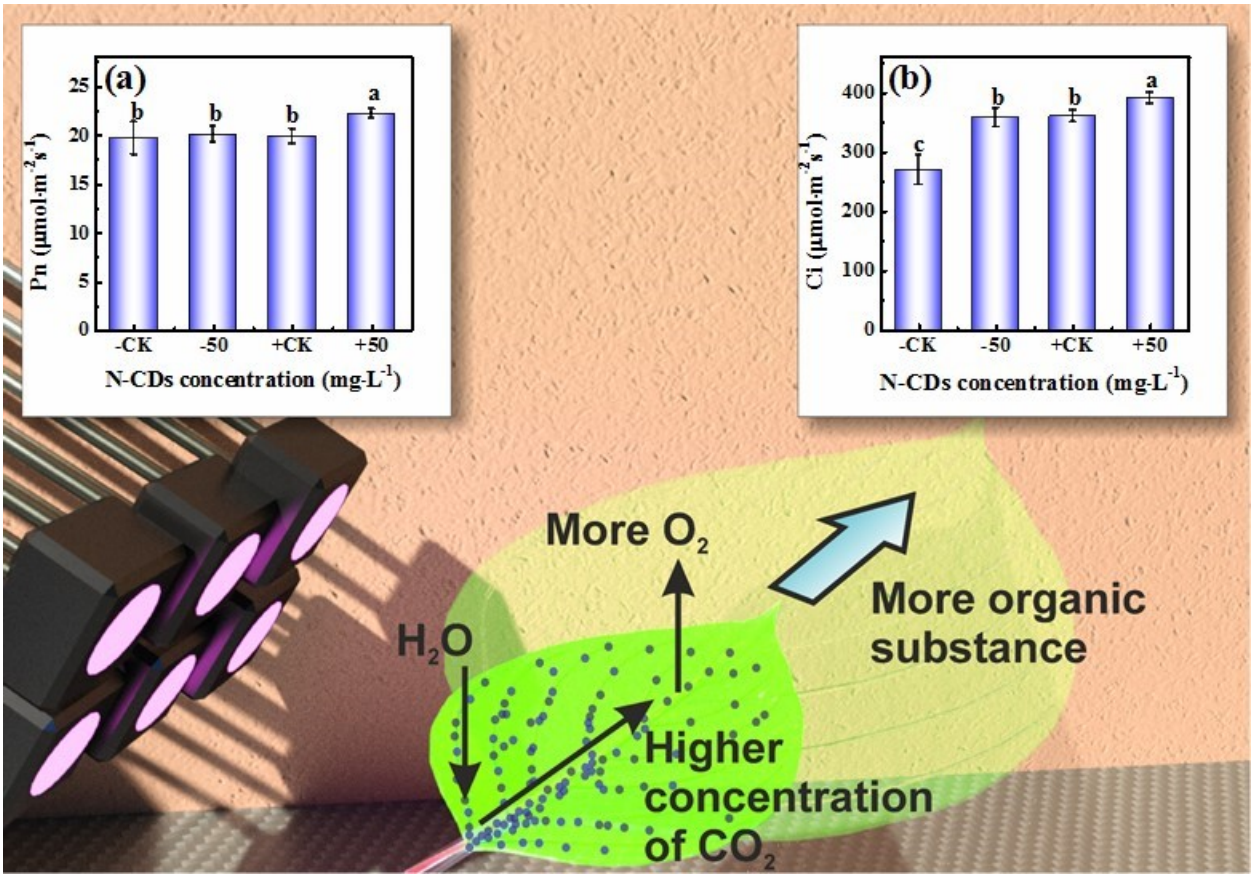

Fig. S11 Diagram of the N−CDs promoting plant photosynthesis (inset: (a) Pn and (b) Ci of the lettuce of the differently treated groups ($p<0.05$, N=4)).

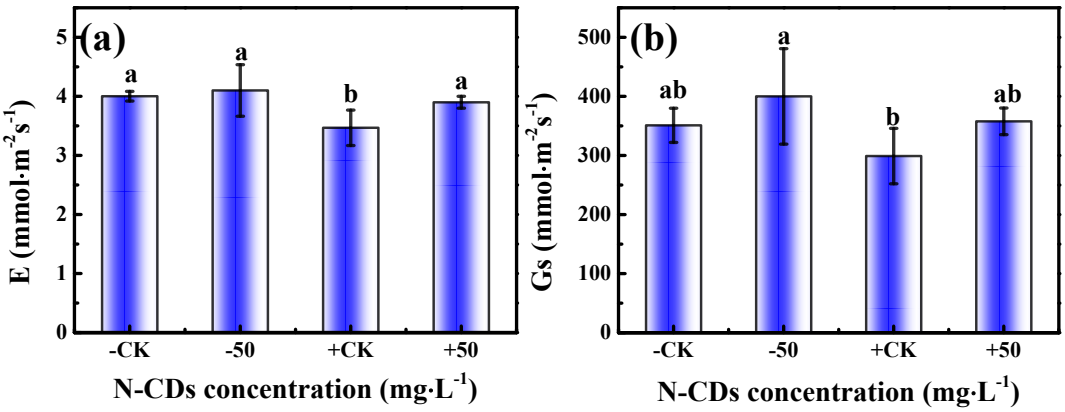

Fig. S12 E and Gs of lettuce with different treatment.

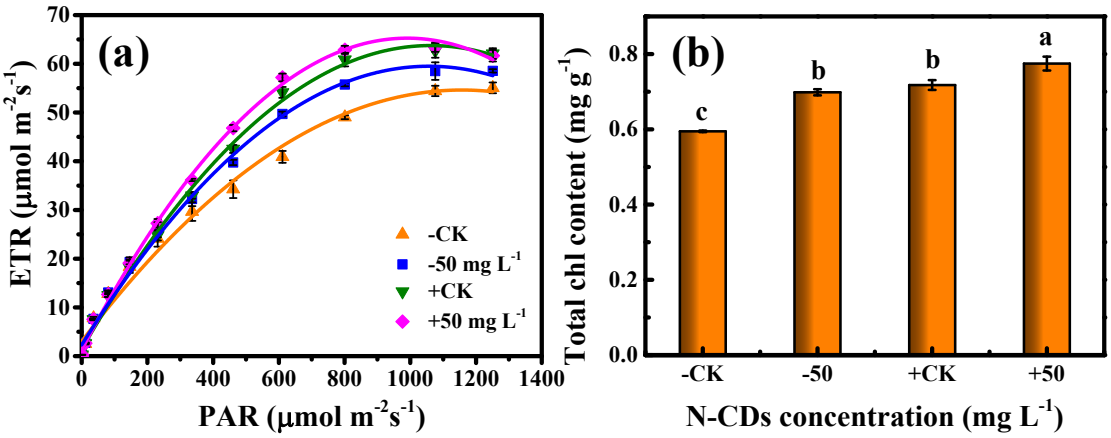

Fig. S13 Repaid photosynthetic light curves (a) and total chlorophyll contents (b) of lettuce with different treatment.

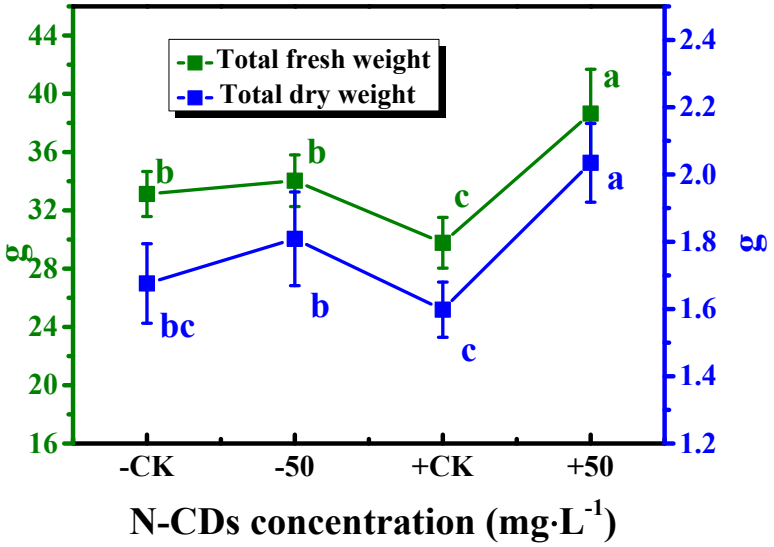

Fig. S14 The fresh and dry weight of lettuce with different treatments ($p<0.05$, $N \geq 6$).

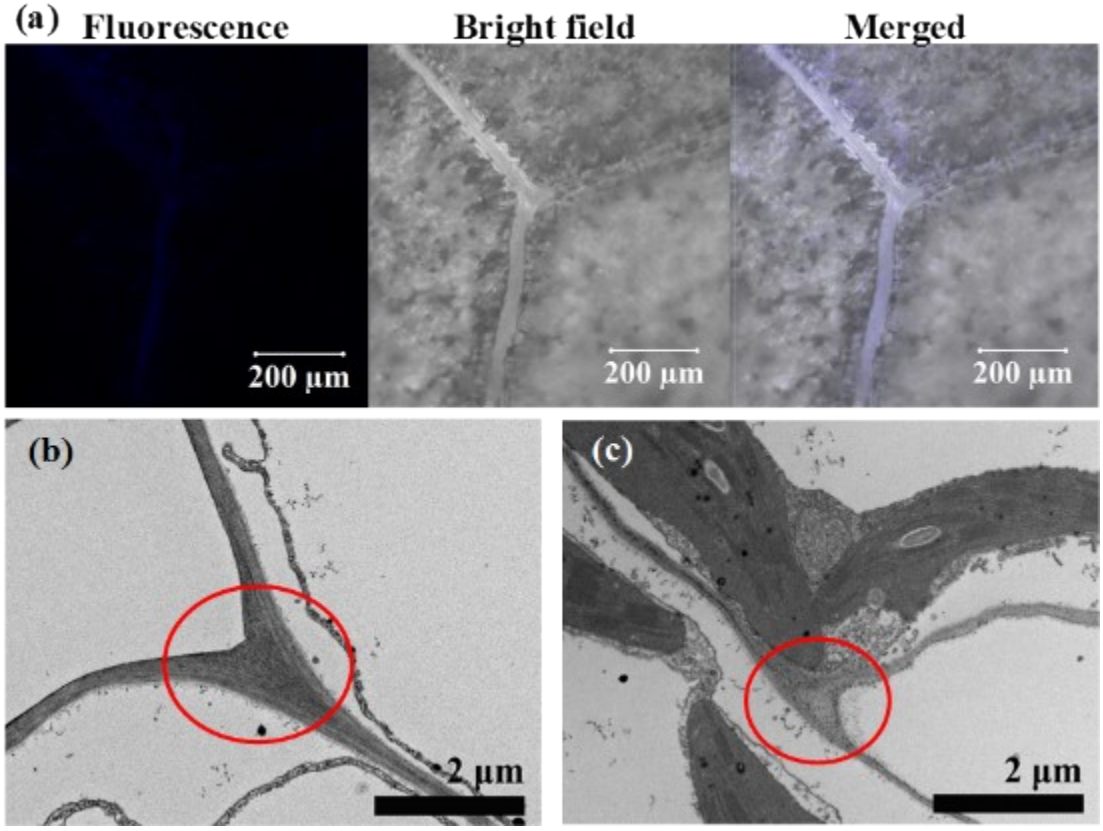

Fig. S15 (a) LSM images of N3−CDs treated leaf. (c) TEM image of leaf with pure water; (d) TEM image of leaf with N3−CDs

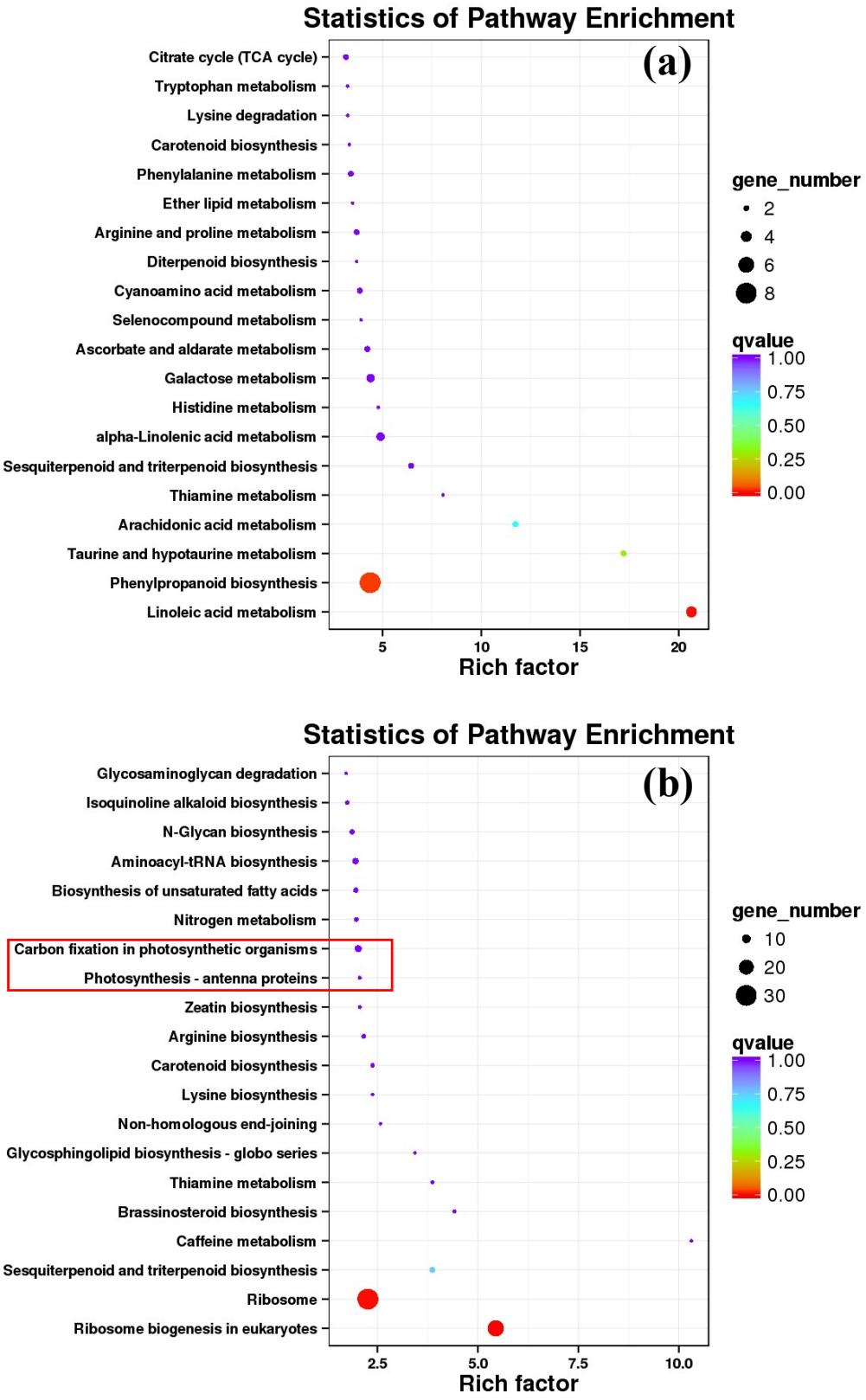

Fig. S16 KEGG enrichment picture: (a) –CK vs −50 and (b) +CK vs +50.

Table S4 Fluorescence lifetime fitted results of N-CDs in H_2O_2 solution (N=3, P. < 0.05, letters difference represents significant differences)

Sample	τ_1 (ns)	A_1	Rel(%)	τ_2 (ns)	A_2	Rel(%)	τ_{ave} (ns)	R^2
IRF(N1-CDs)	0.46	15947.33		2.86	38.74		0.49	0.990
CK(N1-CDs)	3.57±0.09	718.89±11.39	21.92±0.56	13.24±0.16	690.80±18.90	78.08±0.562	11.12±0.97b	1.0770
Dark(N1-CDs)	4.07±0.14	820.42±64.76	26.92±2.19	13.69±0.27	665.45±10.92	73.08±32.90	11.10±0.06b	1.1301
LED(N1-CDs)	3.12±0.20	1052.05±112.52	52.04±4.05	6.84±0.10	439.61±3.77	47.96±4.049	4.91±0.22c	1.1712
LED+UV(N1-CDs)	3.42±0.11	1237.99±69.32	64.17±3.66	7.97±0.24	297.41±32.96	35.83±3.660	5.05±0.03c	1.2224
IRF(N3-CDs)	0.39	478.47		2.57	23.44		0.92	0.987
CK(N3-CDs)	5.44±0.025	699.65±12.70	25.36±0.34	14.48±0.02	771.72±4.70	74.64±1.011	12.19±0.02a	1.1225
Dark(N3-CDs)	5.36±0.097	704.53±15.41	22.52±6.98	14.62±0.16	726.63±16.78	73.79±1.011	11.89±0.56a	1.0208
LED(N3-CDs)	2.97±0.035	1056.40±14.54	53.27±1.06	6.97±0.11	394.76±19.40	46.73±1.062	4.84±0.03c	1.0071
LED+UV(N3-CDs)	3.22±0.169	785.73±579.97	60.05±7.62	6.32±0.37	395.78±82.71	39.95±7.617	4.45±0.02d	1.0491

$$\tau_{average} = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2}$$

Table S5 Fluorescence lifetime fitted results of N-CDs in pure water (N=3, P. < 0.05, letters difference represents significant differences).

Sample	τ_1 (ns)	A_1	τ_2 (ns)	A_2	$\tau_{average}$ (ns)	R^2
IRF(N1-CDs)	0.68	516.01	0.68	516.01	0.68	0.987
Dark(N1-CDs)	4.07±0.41	321.91±52.04	13.10±0.51	61.86±40.03	11.83±0.19a	0.997
LED(N1-CDs)	2.97±0.22	549.57±74.83	6.84±0.36	456.74±57.42	5.50±0.06d	0.997
LED+UV(N1-CDs)	2.91±0.09	553.78±66.32	6.57±0.27	427.30±24.96	5.24±0.06d	0.998
IRF(N3-CDs)	0.89	580.57	0.83	580.57	0.89	0.989
Dark(N3-CDs)	2.63±0.23	376.41±64.91	11.34±0.55	545.87±15.37	10.13±0.26b	0.997
LED(N3-CDs)	3.92±0.29	768.38±26.06	11.66±1.87	187.45±68.85	7.02±0.49c	0.997
LED+UV(N3-CDs)	2.79±0.46	596.21±154.32	6.43±0.92	352.62±142.29	4.79±0.14e	0.997

$$\tau_{average} = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2}$$

Table S6 Gas chromatography results of gases emitted from CDs.

Sample	Light	Time	CO_2 content （%）
5% CO_2 standard	\	1.95	5
CA-CDs	Dark	0	0
(HPR+H_2O_2)	LED	0	0
	LED+Uv	1.99±0.011	0.0254±0.0031
N3-CDs	Dark	1.96±0.024	0.0445±0.0067
(HPR+H2O2)	LED	1.96±0.003	0.0614±0.0086
	LED+Uv	1.97±0.037	0.0528±0.0001
CA-CDs	Dark	0	0
(lettuce extract +H_2O_2)	LED	0	0
	LED+Uv	0	0
N3-CDs	Dark	1.89±0.007	0.0330±0.002
(lettuce extract +H_2O_2)	LED	1.99±0.028	0.0315±0.002
	LED+Uv	1.95±0.045	0.0374 0.004

Table S7 Candidate transcripts related to lettuce photosynthesis

Function	Gene name	Enzyme	KO id
Carbon fixation in photosynthetic organisms	*PEPC*	phosphoenolpyruvate carboxylase	K01595
	MDH2	malate dehydrogenase	K00026
	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	K00134
	ALDO	fructose-bisphosphate aldolase, class I	K01623
Photosynthesis	*psbW*	photosystem II PsbW protein	K02721

References:

(1) Li, Y.; Pan, X.; Xu, X.; Wu, Y.; Zhuang, J.; Zhang, X.; Zhang, H.; Lei, B.; Hu, C.; Liu, Y. Carbon dots as light converter for plant photosynthesis: Augmenting light coverage and quantum yield effect. *J. Hazard. Mater.* **2021**, 410, 124534.

(2) Chandra, S.; Pradhan, S.; Mitra, S.; Patra, P.; Bhattacharya, A.; Pramanik, P.; Goswami, A. High throughput electron transfer from carbon dots to chloroplast: a rationale of enhanced photosynthesis. *Nanoscale* **2014**, 6, 3647-3655.

(3) Li, W.; Wu, S.; Zhang, H.; Zhang, X.; Zhuang, J.; Hu, C.; Liu, Y.; Lei, B.; Ma, L.; Wang, X. Enhanced Biological Photosynthetic Efficiency Using Light‐Harvesting Engineering with Dual‐Emissive Carbon Dots. *Adv. Funct. Mater.* **2017**, 1804004.